



1 (HMB-1)

[illegible]

1) Usual Interstitial Pneumonia; UIP  
 2) Desquamate Interstitial Pneumonia; DIP  
 3) Non-Specific Interstitial Pneumonia; NSIP  
 4) Acute Interstitial Pneumonia; AIP  
 DIP  
 Respiratory Bronchiolitis-associated Interstitial Lung Disease; RB-ILD  
 Idiopathic Pulmonary Fibrosis; IPF  
 UIP  
 IPF  
 γ  
 IPF  
 (1)

High Mobility Group Box Protein; HMB-1  
High Mobility Group Protein; HMG-1  
1964 HMB-1  
GENETYX SOFTWARE DEVELOPMENT  
HMB-1 HMB-1 98.6  
HMB-1 99.1 HMB-1 HMB-1  
81.2 HMB-2 72.3 HMB-2  
79.4

[illegible]

20

30

40

50



HMGB-1 (hypersensitivity pneumonitis; HP) (bronchoalveolar lavage fluid; BALF) HMGB-1 IPF NSIP BALF HMGB-1

HMGB-1 10

HMGB-1 10

Ullrich et al., Proc Natl Acad Sci USA (2002) 99: 12351-6

10

3 13

14 BALF HMGB-1

HMGB-1 p38MAPK NF-κB

in vivo

HMGB-1 20

Palumbo et al., J Cell Biol (2004) 164: 441-9

HMGB-1 (Vascular Endothelial Growth Factor; VEGF) TNFα

8(IL-8) (Ono M et al., Chemother Pharmacol (1999) 43: 569-71; Andersson U et al., J Leukoc Biol (2002) 72: 1084-91)

Schluter et al., Am J Pathol (2005) 166: 1259-63

30

Harada N et al., J Immunol (2005) 175: 1224-31; Burdick MD et al., Am J Respir Crit Care Med (2005) 171: 261-8

Dumitriu et al., J Immunol (2005) 174: 7506-15

HMGB-1 T

IPF HP BALF HMGB-1

HMGB-1 IPF

HMGB-1

HMGB-1

HMGB-1

HMGB-1

1(HMGB-1)

(1)

10

20

30

40

50

50

HMGB-1 TUNEL 14 (a) TUNEL (b) b TUNEL HMGB-1 14 (c) TUNEL (d) a  $c \times 40$  b  $d \times 200$

HMGB-1 (a) 14 HMGB-1  $\pm SE$  (b) 14 HMGB-1 (c) 14 HMGB-1 TUNEL (d)

10

BAL HMGB-1 14 HMGB-1 BAL  $\pm SE$

EP (a) (b) 14 EP BALF HMGB-1 BALF HMGB-1 (c) 14 EP  $\pm SE$  (d) 14 EP

20

BAL EP 14 EP BAL  $\pm SE$

30

HMGB-1 (a) (b) (c) HMGB-1 24 \*p<0.01, +p<0.05

HMGB-1 HMGB-1 TUNEL BALF

40

HMGB-1 HMGB-1 ( ) IgG IgM

HMGB-2 HMGB-1 HMGB-2 HMGB-1

50

HMGB-2 HMGB-1  
 HMGB-1 HMGB-2  
 HMGB-1 HMGB-2  
 HMGB-1 HMGB-2  
 2 HMGB-1 HMGB-2  
 5 HMGB-1 HMGB-2  
 10

HMGB-1 HMGB-2 ELI SA BI ACORE  
 ELI SA BI ACORE

10

HMGB-1 HMGB-2  
 -2 HMGB-1 HMGB-2  
 HMGB-2 HMGB-2  
 HMGB-1 HMGB-2  
 HMGB-1 HMGB-2 ELI SA

HMGB-1  
 HMGB-1 HMGB-1  
 ELI SA BI ACORE

20

HMGB-1  
 HMGB-1

HMGB-1 HMGB-1  
 HMGB-1  
 HMGB-1

30

HMGB-1  
 HMGB-1 HL-60  
 Goodwin H et al., Biochem Biophys Acta (1975) 405: 280-91; Yoshida M et al., J Biochem (1980) 95: 117-24; Adachi Y et al., J Chromatogr (1992) 530: 39-46  
 HMGB-1 HMGB-2  
 HMGB-1

40

HMGB-1  
 HMGB-1 GenBank Accession No. NP\_002119  
 GenBank Accession No. NM\_002128  
 HMGB-1

50

50

10


20

30

40

50

Koehler G et al., Nature (1975)  
 256: 495-7  
 BALB/c  
 0.1  $\mu$ g 5mg 0.1  $\mu$ g mg



10

20

30

40

50

□ □ □ □ □ □

[illegible]

□ □ □ □ □ □

[illegible]


[illegible]

□ □ □ □ □ □

□ □ □ □ □ □

in vitro  
1-59878  
W094/ 25585  
; W093/ 12227 ; W092/ 03918 ; W094/ 02602

□ □ □ □ □ □

4444

HMGB-1 HMGB-1  
 Fv Fab Fab' F(ab')<sub>2</sub> (diabody) Db  
 scFv Fv 1 H V<sub>H</sub>  
 L V<sub>L</sub> V<sub>H</sub>-V<sub>L</sub>  
 3 complementarity determining region CDR  
 V<sub>H</sub>-V<sub>L</sub> 6 CDR  
 1  
 3 CDR Fv  
 1 CDR

3 CDR Fv HMGB-1  
 Fab F(ab) L H CH  
 1 Fab' F(ab') 1  
 H CH1  
 b Fab' - SH F(ab') - SH 1  
 Fab' F(ab')<sub>2</sub> 2 Fab' - SH  
 Co MS et al., J Immunol 1994 152: 2968-76  
 (bi val ent)  
 (Holliger P et al. (1993) Proc Natl Acad Sci USA 1993, 90: 6444-8; EP404,097 ; W093/11161 )  
 2 L (V<sub>L</sub>) H (V<sub>H</sub>)  
 5 V<sub>L</sub> V<sub>H</sub>  
 2  
 scFv V<sub>H</sub> V<sub>L</sub> Fv V<sub>H</sub> V<sub>L</sub>  
 scFv Huston JS et al., Proc Natl Acad Sci USA (1988) 85: 5879-83  
 Pluckthun The Pharmacology of Monoclonal Antibodies Vol. 113 Rosenberg and Moore ed (Springer Verlag, New York) pp. 269-315, 1994  
 SCFV DNA  
 (1) H H V DNA L L V DNA  
 DNA PCR  
 (2) DNA H L  
 SCFV DNA  
 SCFV  
 IgG  
 IgG  
 hybrid hybrida (quadrona) Milstein C et al. (1983) Nature 305: 537-40  
 IgG L H  
 4 H CH3 H

10

20

30

40

50

Ridgway JB et al. (1996) Protein Engineering 9: 617-21; Merchant AM et al. (1998) Nat Biotech 16: 677-81

CDR<sub>H</sub> CDR<sub>L</sub> CH1 L PCR  
 DNA DNA  
 V DNA (C<sub>H</sub>)  
 DNA  
 DNA  
 H L DNA  
 DNA H L W  
 O94/ 11523  
 W093/ 12227, W092/ 03918 W094/ 02602, W094/ 25585 W096/ 34096, W096/ 33735  
 reshaped  
 CDR<sub>H</sub> CDR<sub>L</sub> CDR<sub>H</sub> CDR<sub>L</sub>  
 framework region FR DNA  
 DNA PCR DNA  
 DNA DNA  
 EP 239400; W096/ 02576  
 CDR<sub>H</sub> FR CDR<sub>H</sub> FR CDR<sub>L</sub> FR CDR<sub>L</sub>  
 Sato K et al. (1993) Cancer Res 53: 851-6  
 FR W099/ 51743

10

20

30

40

50

50

\_\_\_\_\_

SDS  
 Antibodies : A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory, 1988  
 A  
 G  
 L

□ □ □ □ □ □ □ □ □ □ / □ □ □

□ □ □ □ □ □

□ □ □ □ □ □

93/17706

1111

1. HMG-1 (6) ( : ) ( Wen et al., Nucleic Acids Res (1989) 17: 1197-214)

2 HMB-1  
(T. P. Hopp et al., Proc Natl Acad Sci USA (1981) 78: 3824-8)

3 (M Yoshida et al., J Biol Chem (1992) 267: 6641-5) HMGB-1 HMGB-2  
HMGB-1 HMGB-2

4 1 HMGB-1 167

10

20

30

40

50

180 Lys Pro Asp Ala  
Ala Lys Lys Gly Val Val Lys Ala Glu Lys : 1 HM  
GB-1 Lys Pro Asp Ala Ala Lys Lys Gly Val Val Lys Ala Glu Lys  
: 1 HMGB-2 Lys Ser Glu Ala Gly Lys  
Lys Gly Pro Gly Arg Pro Thr Gly : 2 9

1 Cys Lys Pro Asp Ala Ala Lys Lys Gly Val Val Lys Ala  
Glu Lys : 3

10

(Applied Biosystems) 430A (Model 430A peptide synthesizer) t-  
Cys Lys Pro Asp Ala Ala Lys Lys Gly Val Val  
Lys Ala Glu Lys : 3

p- m

2N

1-X2(DOWEX 1-X2)

(ODS)

20

(HPLC)

HPLC

HPLC ODS YMC-D

-ODS-5 20mmx 300mm TW NCLE GP

-A40 0.1 (TFA) 0 70

7.0mL UVIDEC-100V (210nm 1.

28AUS)

HPLC ODS

YMC-R-ODS-5 4.9mmx 300mm TW NCLE

30

GP-A40 0.1 (TFA) 0

70 1.0mL 25 UVIDEC-10

0V (210nm 1.28AUS)

100

(KLH) (BSA) 10mg 10mM

(pH7.0) N, N- 2.5

- (MBS) 150μ L

40

30 4°C 10mM

(pH7.0) G-2

5(Sephadex G-25) 280nm

MBS- MBS-

pH7.0 2 Cys Lys Pro Asp Ala Ala

Lys Lys Gly Val Val Lys Ala Glu Lys : 3 150

3

HMGB-1 HMGB-2

50

HMGB-1 : 4 HMGB-2 : 5  
 (C. Sanders et al., BBRC (1977) 78: 1034-42)  
 1 500g 140mM 0.5mM PMSF 600mL

2  
 3 140mM 0.5mM PMSF  
 2

4 0.75M 300mL 0.75M 400mL

10

5 0.75M 1,000mL 4

6 3,500mL 21mL  
 2,500mL

20

7

HMGB-1 HMGB-2 20mg

8 HMGB-1 HMGB-2 200mM 7.5 mM (pH9.0) 10mL 200mM 7.5mM (pH9.0)

9 7.5mM (pH9.0) CM- C25 200mM 7.5mM (pH9.0)

10 15 SDS- 1  
 A B HMGB-1  
 C D HMGB-2

30

11 1 A B  
 C D

HMGB-1 HMGB-2

HMGB-1 : 6 HMGB-2 : 7 (P. Cabart et al. Cell Biochemistry and Function 13; 125-133: 1995)

1 HL60 cell 300mL 10% FCS ( ) RPM 1640 ( )

40

2 HL60 RPM 1640 3L PFHM-II ( )

3 PBS Heparin-Sepharose ( )

4 PBS 0.5 M PBS 280nm 5mM (pH9.0) 0.2M

7.5mM (pH9.0) CM- Sephadex C25 (Pharmacia) 200mM 7.5mM  
 pH9.0 4

50

50

50



[illegible]

3-7 El A 415nm

4 12

□ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ ( 1 gY ) □ □ □ □

□ 5□ □ □ 10mL □ TBS(0.14M NaCl, 0.01M Tris/HCl, pH7.4, 0.01%Na<sub>3</sub>) 40mL □ □ □ □ □  
□ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □

[illegible]

7 20g 30  
10mL TBS PBS PBS

8 2

8-10 20 10mg 2g CNBr-

8-2 (7)

8-300.1M pH3

8-4

[illegible]

9 HMB-1 HMB-1 HMB-2

[illegible]

HMGB-1

9 HMG-1, 2

[illegible]

9

□ 1 □ □ □ 5 □ □ □ □ □ HMBG-1(1mg/mL) □ □ HMBG-2(1mg/mL) □ 1: 1 □ □ □ □ □ □ □  
□ □ □ □ □ □ □ □ 1: 1 □ □ □ □ □

2 15% SDS-

pH8.8 20mA 180

3 (2) 0.05% Tween20 20mL 10% BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10  
 5 0.05% Tween20 20mL 10% BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10  
 5 0.05% Tween20 20mL 10% BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10

4 1 BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10

5 0.05% Tween20 20mL 10% BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10

6 9 80μg 20mL 1% BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10

7 (6) 20mL 10% BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10

8 IgG 3 BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10

9 20mL 10% BSA 5.5  
 9mM 1.47mM 137mM 2.68mM (pH7.2) 20mL 4°C 10

10 0.025% 3, 3' - 0.01% 20mL (9) 15

9 30

1 30

1 9 IgY (Up-Data)

3 6 HMGB-1 2 3

2 HMGB-1 3

HMGB-2 40

1 9 HMGB-1

HMGB-2 9

HMGB-1 HMGB-2

5 HMGB-1

1. 50

50 0.9  
 8 BALB/c ( )  
 0.5mL 2 100μ g/mL  
 0.5mL 2  
 ELISA ELISA  
 6 1 ELISA (1)  
 18  
 800μ g/mL 5  
 HMGB-1 0.5mL 3  
 0

( 1 ) ELI SA□

HMGB-1 1μg/mL 100μL 37°C 20 Tween20 0.05% 20mM 5.59mM 1.47mM 137mM 2.68mM pH7.2 1% BSA 10mM - pH7.2 1% 300μL 37°C 2% 100μL 900μL 1000 10000 100000 100μL 37°C 2% 1% BSA 0.1M 100μL 37°C 2% POD IgG (3% BSA 5000 100μL 37°C 2% 3mM 2, 2' - (3- -6- (ABTS) 50mM -24mM 1mL 2μL 1.7 1 15 1 150μL 6N EI A 415nm

[illegible]

BALB/ c P3- X63- Ag8- U1 9085 RPM 16 40 200mL 8

3. ☐ ☐ ☐ ☐

1. RPM 1640 P3- X63- Ag8- U1 50 RPM 1640 50 RPM 1640

10

20

## 30

40

50

6. □ □ □ □ □ □ □ □ □ □ □ □

(1)    □ □ □ □ □ □ □ □ □ □ | gG □ □ □

5. 10mL 22°C 1. 8g 22°C 7000g 15 30mM 40mM pH8.0 2mL 30mM 40mM pH8.0 1000g 20 30 M 40mM pH8.0 DEAE- 1× 10cm 0.4mL 2mL G I gG 280nm 2mL - CL-4B -

(2)            IgM

5. 10mL 20mM (pH7.5) 0.8M  
 20mM (pH7.5) 0.8M  
 Hi Trap IgM purification HP 1mL ( ) 20mM  
 (pH7.5) 0.8M 20mM (pH7.5)  
 IgM

HMGB-1, 2

[illegible]

50 1:1 HMGB-1 (1mg/mL) HMGB-2 (1mg/mL) 1:1 15% SDS pH8.8 20mA 180 - 9cmx 9cm 48mM 39mM 0.0357 W V SDS 20 V V 60mA 2

1 BSA 5.59mM

(American Physiological Society) 7 8 C57B1/6 ( ) KBT ( ) 20 25g ( Schering-Plough, ) 1kg 3U 50μ L ( )

50

0.1 g Y HMB-1 6  
 0.1 g Y ( )  
 (Kim J Y et al., Am J Physiol Lung Cell Mol Physiol (2005) 288: L958-65) HMB-1 (200 μg/ ) 5  
 Y (200 μg/ ) 5  
 (Ulloa L et al., Proc Natl Acad Sci USA (2002) 99: 12351-6) (EP) (40 mg/kg) 3 13 14  
 10  
 -80°C

3. □ □ □ □ □ □ □ (BAL) □ □

BAL (Kuwano K et al., Chest (2000) 118: 451-8)  
 BAL 150mL  
 1mL  
 2  
 Diff-Quick (Baxter Diagnostics)  
 20  
 BAL HMB-1  
 -80°C

4. HMGB-1 □ □ □ □ □ □ □ □

$3 \times 10^{-6} \text{ m}$

SAB-PO

-

30

HMB-1 (Upstate, Lake Placid, NY) 4°C

5. □ □ □ □ □ □ □ □ □ □ □

[illegible][illegible]

TUNEL (Kuwano K et al., J Clin Invest (1999) 104: 13-9; Maeyama T et al., Am J Physiol Lung Cell Mol Physiol (2001) 280: L1128-37; Tsuburai T et al., Hum Gene Ther (2002) 13: 1945-60) DNA DeadEnd Colorimetric Apoptosis Detection System (Promega, M, USA) TUNEL (Kuwano K et al., Lab Invest (2002) 82: 1695-706) 200 20 TUNEL

## 7. HMGB-1 ☐ ☐ ☐ ☐ ☐ ELISA

□ □ □ □ □ HMBG-1 □ □ □ □ □ □ □ □ □ □ □ □ □ □ HMBG-1 □ □ □ □ ELI SA □ □  
□ □ (Yamada S et al., Clin Chem (2003) 49: 1535-7) □ HMBG-1 □ □ □ □ □ □ □ 2μ g/L  
□ □ □ □ □  
□ □ □ □ □ □

8.                     

                    Sircor Collagen Assay (Biochemical, Northern Ireland, UK) (Kitani A et al., J Exp Med (2003) 198: 1179-88)

9.           

BALF BAL TUNEL ELISA (analysis of variance; ANOVA) Scheffe F Kruskal-Wallis Mann-Whitney U 0.05 P StatView J-4.5 (Abacus Concepts Inc., Berkeley, CA)

1.           HMGB-1                    BALF

HMGB-1 ( $\pm$  SEM) IPF  $3.7 \pm 1.9$  NSIP  $0.1 \pm 0.1$  CVD-IP  $0.8 \pm 0.5$  HP  $4.1 \pm 3.4$   $0.5 \pm 0.3$  ng/mL HMGB-1 (5) BALF HMGB-1 ( $\pm$  SEM) IPF  $3.5 \pm 0.6$  NSIP  $4.9 \pm 2.4$  CVD-IP  $2.2 \pm 0.5$  HP  $9.4 \pm 4.2$   $1.6 \pm 0.4$  ng/mL BALF HMGB-1 IPF HP ( $p < 0.05$   $p < 0.01$ ) (6)

2. IPF NSIP                    HMGB-1

HMGB-1 NSIP IPF (7)

3.                     HMGB-1

HMGB-1 HMGB-1 1 5 7 14 14 (8)

4.                     HMGB-1

14 HMGB-1 (10a)

7 14 HMGB-1 14 (9a c) HMGB01 14 (10b)

TUNEL 14 DNA (9b) HMGB-1 14 TUNEL (9d 10c)

HMGB-1 (10d)

BALF BALF 14  
HMGB-1 BALF  
14 BALF (11)

5. 14

14 (12c)

HMGB-1 BALF HMGB-1 (12a b)  
14 (12d)

BALF EP BALF 14 (13)

HMGB-1

(a) (c)

W-38 DS Pharma Biomedical (Osaka, Japan) W-38 75-cm<sup>2</sup> (FALCON, Franklin Lakes, NJ) 10% FBS MEM 37°C 5% HMGB-1 10 30 ng/mL 24 W-38

W-38 / EDTA

Annexin-V-FLUOS staining kit (Roche Diagnostics, Penzberg, Germany)

$1 \times 10^6$  PBS Annexin V-FITC Propidium iodide (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 5 mM CaCl<sub>2</sub>) 20 Coulter EPICS XL flow cytometer (Coulter, Miami, FL)

W-38 -80°C Sircor Collagen Assay kit (Bioroll, Northern Ireland, UK)

(b)

W-38 DS Pharma Biomedical (Osaka, Japan) W-38 96 10% FBS MEM 37°C 5% HMGB-1 10 30 ng/mL 24 W-38 Tetra color ONE assay kit (SEIKAGAKU CORPORATION, Tokyo, Japan) W-38 HMGB-1 N=8 tetra color ONE 37°C 4 (Easy Reader EAR 340; SLT-Lab instruments, Austria) 540 nm

10

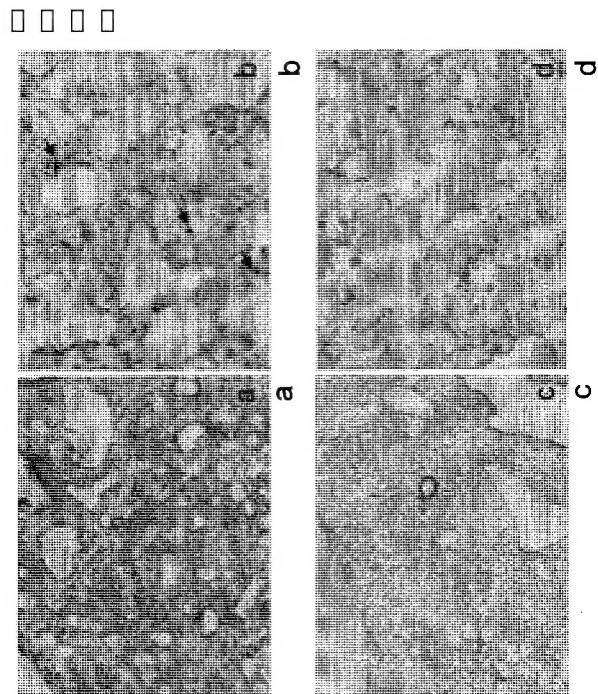
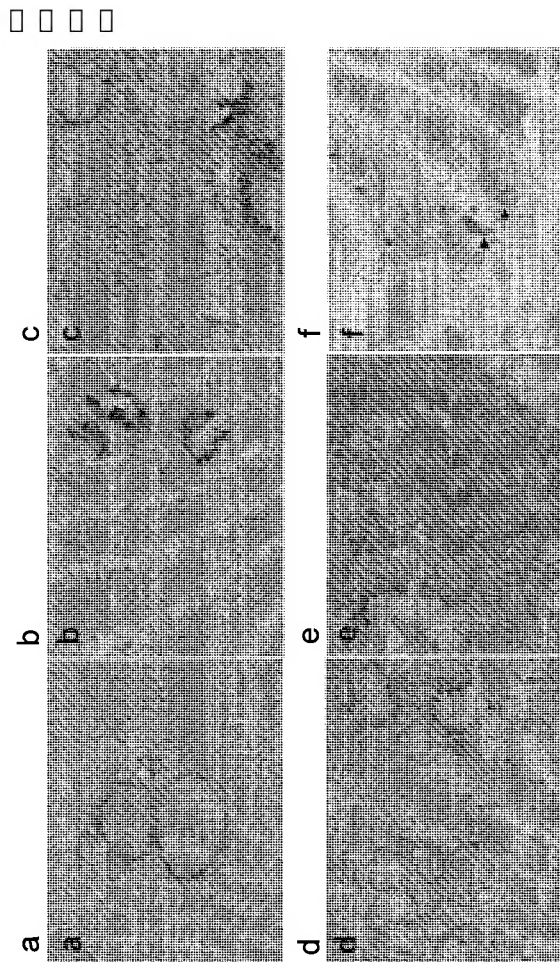
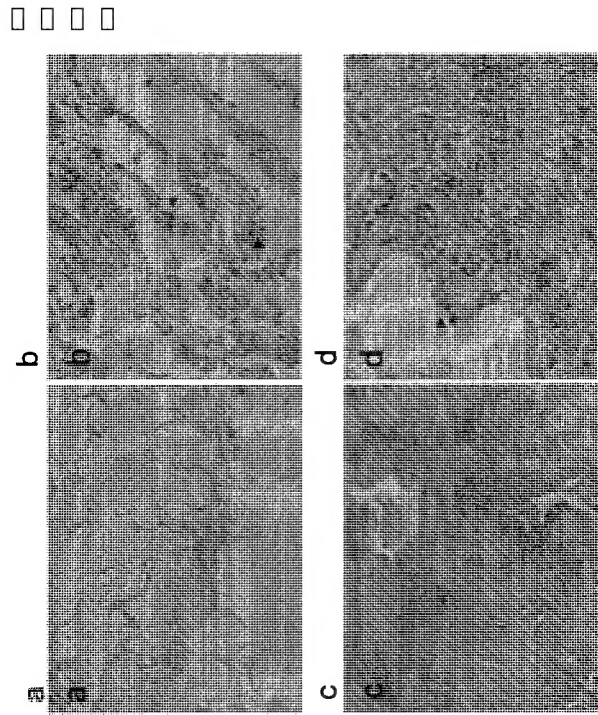
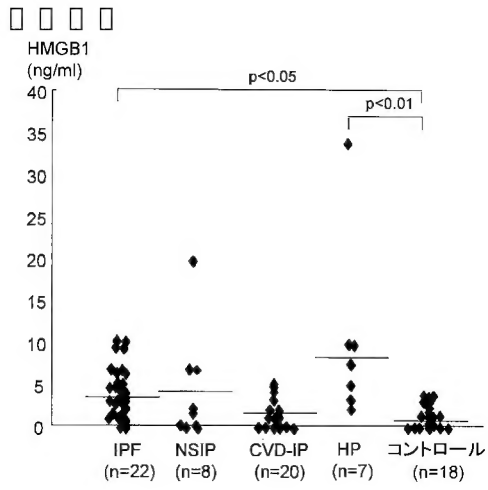
20

30

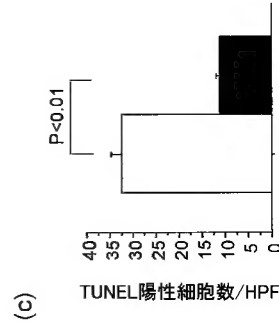
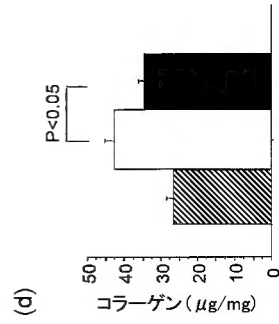
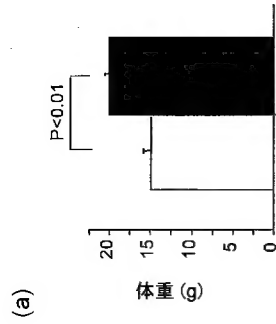
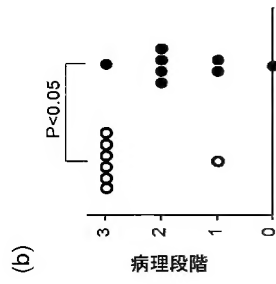
40

50

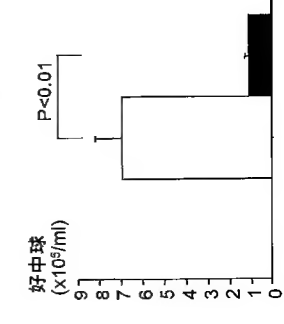
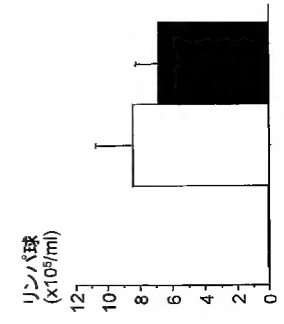
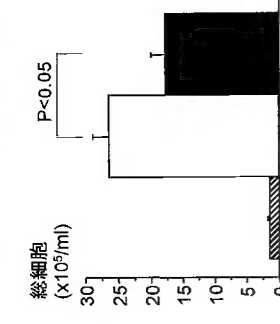
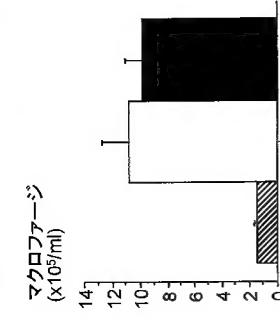




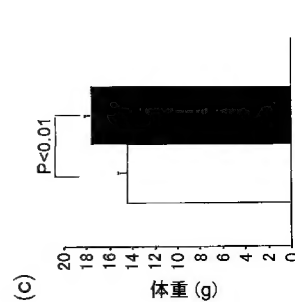
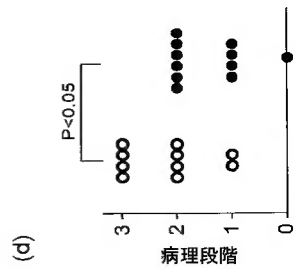
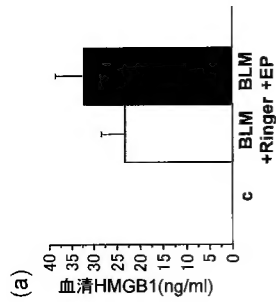
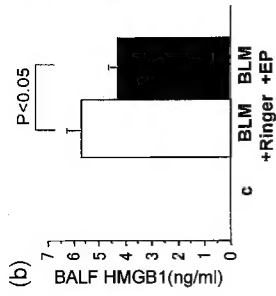
□ □ □ □ □



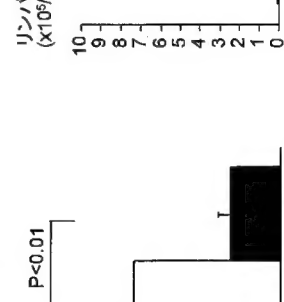
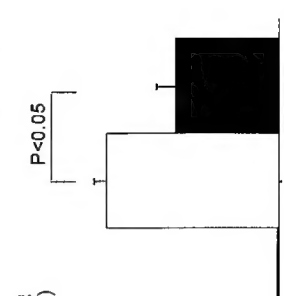
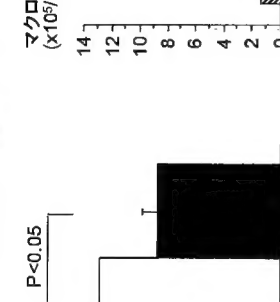
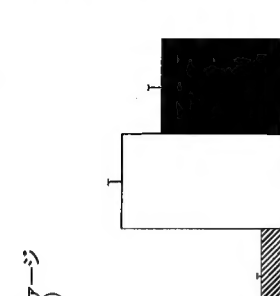
□ □ □ □ □



□ □ □ □ □

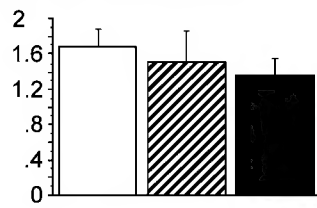


□ □ □ □ □

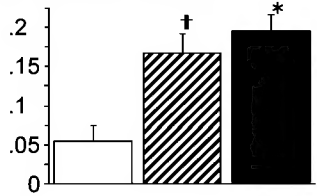


□ □ □ □ □

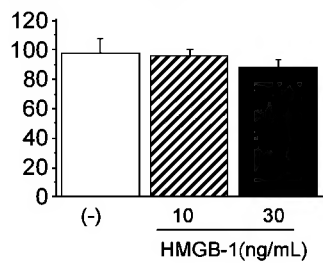
(a) アポトーシス細胞 (%)



(b) 生存細胞 (吸光度)



(c) コラーゲン (mg/ml)



□ □ □ □ □

0005241518000001. app

□ □ □ □ □ □ □ □ □ □ □ □ - □ □ - □ □    □ □ □ □ □ □ □ □ □ □ □ □

□ □ □ □ □ □ □ □ □ □ □ □ Vol . 10, Nb. 3 □ p. 204-208

□ □